ES:CJ-11 (from p 20)

EXHIBIT B 028

CJ 11.1 Good expansion of "flat" ES-like colonies; however endoderm differentiation taking place more abundantly. Only clone w/ good feeders (ie no over-growth)

CJ 11.2 Over-grown by feeders that were not properly inactivated. Very few ES-like colonies recognizable

CJ 11.3 Also contained w/ non-inactivated STO cells. Some very good colonies remain though.

CJ 11.4 Mostly endoderm; a few ES-like colonies.

① Added Ca^{++}/Mg^{2+} free PBS to each of above; chose individual colonies, trypsin 5' → chose individual cells → 96 well plates w/ 3T3 cells.
did 2 of 8 / Line [half has bFGF & h Steel factor (4 singles)]
→ 5% CO_2 / 5% O_2
N:D manipulations took a long time & pH in medium changed. (expect poor cloning efficiency if any)

② Split CJ 11.1 → T25 w/ 3T3 cells.

→ Will try to pan off fibroblasts/endoderm by allowing split & free cells to attach & keep floated after 15-30 minutes

4 x T75 = 2 T75
2 x T25 = 1.5 T25
[10 wells]
[5 + 4]

① bFGF + h Steel factor 96 well plates: No colonies.
② ES 96 well plate ② colonies CJ 11.1, 3, 4
① colony CJ 11.2
Added new 3T3 cells w/o moving colonies.

③ Split CJ 11.1 → 2 T75's w/ 3T3 cells old 6/6/97
Some endoderm differentiation. (bad in one flask)

④ Individual colonies w/o feeders of CJ 11.3 & CJ 11.4 still undifferentiated.

⑤ "Panning" did not work the 6 well plate of CJ 11.3 was very over-grown. However, Many good colonies remained. AP Rxn (Donovan, 1986) ④ !!!!!!!